

Cholesterol sulfate in human physiology: what's it all about?

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Abstract Cholesterol sulfate is quantitatively the most important known sterol sulfate in human plasma, where it is present in a concentration that overlaps that of the other abundant circulating steroid sulfate, dehydroepiandrosterone (DHEA) sulfate. Although these sulfolipids have similar production and metabolic clearance rates, they arise from distinct sources and are metabolized by different pathways. While the function of DHEA sulfate remains an enigma, cholesterol sulfate has emerged as an important regulatory molecule. Cholesterol sulfate is a component of cell membranes where it has a stabilizing role, e.g., protecting erythrocytes from osmotic lysis and regulating sperm capacitation. It is present in platelet membranes where it supports platelet adhesion. Cholesterol sulfate can regulate the activity of serine proteases, e.g., those involved in blood clotting, fibrinolysis, and epidermal cell adhesion. As a result of its ability to regulate the activity of selective protein kinase C isoforms and modulate the specificity of phosphatidylinositol 3-kinase, cholesterol sulfate is involved in signal transduction. Cholesterol sulfate functions in keratinocyte differentiation, inducing genes that encode for key components involved in development of the barrier. The accumulating evidence demonstrating a regulatory function for cholesterol sulfate appears solid; the challenge now is to work out the molecular mechanisms whereby this interesting molecule carries out its various roles.—Strott, C. A., and Y. Higashi. Cholesterol sulfate in human physiology: what's it all about? *J. Lipid Res.* 2003. 44: 1268–1278.

Supplementary key words sterol • sulfonation sulfoconjugation • sulfotransferase • signal transduction

The biotransformation of molecules by sulfonation is a basic and pervasive metabolic pathway of primary importance. Sulfonated compounds comprise a remarkable array of substances from macromolecular molecules such as glycosaminoglycans, proteoglycans, and secretory and membrane proteins to small, endogenous molecules such as neurotransmitters and hormones (1, 2). Furthermore, sulfonation is important in the metabolism of drugs and xenobiotics (3). Sulfoglycolipids such as sphingolipids and galactoglycerolipids are abundant in myelin as well as spermatozoa, kidney, and small intestine (4), and have been

implicated in a variety of physiologic functions through their interactions with extracellular matrix proteins, cellular adhesive receptors, blood coagulation systems, complement activation systems, and cation transport systems (5). Sulfonation has a significant role in the biotransformation of many endogenous low-molecular-weight compounds, including catecholamines (6), iodothyronines (7), and vitamin C (8). Likewise, sulfonation is an important modification of cholesterol (9) and its derivatives, bile acids (10), vitamin D (11, 12), and steroids (13).

Although the sulfoconjugation of steroids was first described in the late 1930s and early 1940s [see Siiteri (14) and references cited therein], the sulfoconjugation of cholesterol, the immediate precursor of all steroids, was not appreciated until much later, when the seminal report on the isolation of cholesterol sulfate from a natural source appeared in 1964 (15). While the initial report described the isolation of cholesterol sulfate from the bovine adrenal gland, it was subsequently isolated from diverse human sources and tissues (16–18).

The discovery of the existence of cholesterol sulfate was followed by a concerted effort to discern its biologic import. Initial interest centered on cholesterol sulfate serving as a substrate for the synthesis of sulfonated steroids (19–22). Eventually, additional actions of cholesterol sulfate were considered and explored. For example, effects on cholesterol synthesis (23), sperm capacitation (24), and the activity of thrombin and plasmin (25) were reported. Of particular interest was the finding that cholesterol sulfate can function as a regulatory molecule (26, 27). One of the most investigated physiologic roles for cholesterol sulfate has been in keratinocyte differentiation and development of the epidermal barrier (28, 29). In brief, it is now evident that cholesterol sulfate, akin to certain other steroid sulfates such as pregnenolone sulfate (30), is considerably more than a simple metabolic end product “whose sole fate is removal by the excreta” (9).

Abbreviations: DHEA, dehydroepiandrosterone; MCR, metabolic clearance rate; P₄₅₀, cytochrome P₄₅₀; PR, production rate; [S], plasma steroid/sterol concentration; SCC, side-chain cleavage; SULT, cytosolic sulfotransferase.

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Thus, the aim of this review is to bring the reader up to date on the status of this interesting molecule as well as point to the future and suggest possible new directions.

PLASMA LEVELS

Cholesterol sulfate was first isolated from human plasma in 1965 and found to be present in a concentration of 300 $\mu\text{g}/100\text{ ml}$ (16). The apparent validity of this initial value was soon confirmed by reports of plasma cholesterol sulfate levels involving a limited number of subjects that ranged from 174 to 328 $\mu\text{g}/100\text{ ml}$ (31–33). Studies involving larger groups of subjects but utilizing different methodologies revealed plasma cholesterol sulfate levels in normal subjects of 150–250 $\mu\text{g}/\text{ml}$ (34, 35), $178 \pm 31\text{ }\mu\text{g}/100\text{ ml}$ (men and women) (36), 134–322 $\mu\text{g}/\text{ml}$ (men) and 117–214 $\mu\text{g}/\text{ml}$ (women) (37), 118–138 $\mu\text{g}/\text{ml}$ (men) (38), $272 \pm 64\text{ }\mu\text{g}/\text{ml}$ (men) and $175 \pm 70\text{ }\mu\text{g}/\text{ml}$ (women) (39), $134 \pm 29\text{ }\mu\text{g}/\text{ml}$ (men) (40), and $253 \pm 25\text{ }\mu\text{g}/\text{ml}$ (women) (41).

Plasma cholesterol sulfate, which is carried in part by LDLs (35, 42, 43), can be significantly elevated in certain pathologic conditions, such as cirrhosis of the liver (41), hypercholesterolemia (36, 41), and hypothyroidism (44). In recessive X-linked ichthyosis (but not other forms of ichthyosis), a skin disorder characterized by excessive scaling, the levels of plasma cholesterol sulfate are strikingly elevated, often by more than an order of magnitude (34, 35, 39); furthermore, the high plasma levels are associated with elevated levels of cholesterol sulfate in the stratum corneum of the epidermis (45). On the other hand, plasma dehydroepiandrosterone (DHEA) sulfate, which in young adults is normally as abundant in the circulation as cholesterol sulfate, is not elevated in recessive X-linked ichthyosis (34, 35, 38, 39). The latter finding clearly suggests that the primary metabolic pathways for these two prominent circulating steroid sulfoconjugates are dissimilar (34).

There is another important distinction between these two sulfolipids that relates to age. As noted, cholesterol sulfate and DHEA sulfate are two of the most abundant steroid sulfates in the human circulation where their concentrations overlap, although the mean level of the latter sulfoconjugate tends to be somewhat higher (34, 37–39). There is, however, a remarkable age-dependent decrease in plasma DHEA sulfate from its postpubertal peak (46, 47), whereas there appears to be no age-dependent change in the level of plasma cholesterol sulfate (37).

Finally, plasma cholesterol sulfate is modestly but significantly increased during the course of normal pregnancy, an increase associated with its elevated placental production (48). While this is an interesting observation, the physiologic import of this finding is presently obscure.

TISSUE DISTRIBUTION

Cholesterol sulfate in humans is found in urine, bile, and feces (17, 32, 49, 50), in addition to its presence in the circulation; it is also a normal constituent in seminal

plasma as well as spermatozoa (24, 51). Cholesterol sulfate is present in platelets (52, 53) and red blood cells (33, 34, 37, 44, 54); it is also a normal constituent in a variety of human tissues including skin, hair, nails, aorta, adrenal, liver, and kidney (17, 18). In other species, such as the rat, cholesterol sulfate is also widely distributed, with kidney, spleen, lung, adrenal, and gonad containing the highest amounts (55).

In skin, cholesterol sulfate is located predominantly in the epidermis where it functions as an important regulatory molecule during formation of the barrier (45, 56–58). Cholesterol sulfate is formed in the basal and spinous layers of the epidermis, reaches its highest concentration in the granular layer, and then decreases in the stratum corneum as a result of the action of steroid sulfate sulfatase (58, 59). Cholesterol thus starts to undergo sulfoconjugation in the lower reaches of the epidermis and, as keratinocytes, drop out of the cell cycle, begin the process of differentiation, and migrate into the outer layer of living epidermal cells where the amount of cholesterol sulfate reaches a maximum. It is then hydrolyzed in the dead layer of skin, the stratum corneum, creating what has been referred to as “the epidermal cholesterol sulfate cycle” (57). In blood and gastrointestinal epithelia, cholesterol sulfate is a minor sterol constituent, and the cholesterol-cholesterol sulfate ratio approximates 500:1, whereas in the stratum corneum of the normal epidermis the ratio is 10:1 to 5:1 (60). However, in recessive X-linked ichthyosis, which is caused by steroid sulfatase deficiency, the ratio of cholesterol-cholesterol sulfate in the stratum corneum can be as low as 1:1 (60). Cholesterol sulfate is a normal constituent of hair and nails (61, 62); furthermore, similar to its elevation in the stratum corneum, the level of cholesterol sulfate in hair and nails is also significantly increased in recessive X-linked ichthyosis (62).

SYNTHESIS AND METABOLISM

Production rates (PRs) of cholesterol sulfate have not been evaluated extensively, but, based on estimated metabolic clearance rates (MCRs) and plasma steroid/sterol concentrations ($[\text{S}]$), PRs ($\text{PR} = \text{MCR} \times [\text{S}]$) of 35 mg/day and 163 mg/day were determined for two normal subjects (31). Interestingly, the PR for cholesterol sulfate appears to be comparable to the PR of $\sim 45\text{ mg/day}$ determined for the other prominent circulating steroid sulfate, i.e., DHEA sulfate. The latter PR was calculated for young men from reported DHEA $[\text{S}]$ and MCR values (47, 63). It should be noted that, in contrast to their unconjugated counterparts, cholesterol sulfate and DHEA sulfate are cleared slowly from the circulation with MCRs, respectively, of 10–17 l/day and 15–30 l/day (31, 64). The slow clearance of these steroid sulfoconjugates from the circulation reflects their tight binding to plasma proteins, especially albumin (65). Importantly, although the sulfoconjugates of cholesterol and DHEA have similar PRs, they are, nevertheless, derived from different sources and have different metabolic routes. That is, DHEA sulfate is derived

principally from the adrenal cortex (66, 67), specifically the zona reticularis (68), whereas cholesterol sulfate appears to be formed in many tissues, although relative tissue contributions to total production are not known. Nevertheless, one could speculate that based on the size of skin and the knowledge that cholesterol sulfate is a significant lipid constituent of the epidermis, a major portion of the total body production of cholesterol sulfate may be derived from this source. The striking elevation in plasma cholesterol sulfate that occurs in recessive X-linked ichthyosis, as noted previously, is consistent with this conclusion.

Cholesterol sulfate is present in urine and feces, indicating that both excretory routes are utilized (17, 32, 49, 50). Nonetheless, a similar argument as used above suggesting that skin is a major source of total body cholesterol sulfate production can be used to further suggest that skin might also be an important route whereby cholesterol sulfate is lost from the body. That is, because of the large extent of skin and the presence of substantial epidermal cholesterol sulfate, a significant amount of cholesterol sulfate must necessarily be lost via the normal desquamation process (69).

CHOLESTEROL SULFOTRANSFERASES

Cytosolic sulfotransferases (SULTs) constitute a superfamily of enzymes that catalyze the sulfoconjugation of relatively small endogenous compounds such as hormones and neurotransmitters, as well as drugs and xenobiotics (3). The SULT superfamily is divided into five families,

one of which (SULT2) is primarily engaged in the sulfoconjugation of neutral steroids and sterols. The human SULT2 family is further divided into two subfamilies, i.e., SULT2A1 and SULT2B1 (70). Additionally, the SULT2B1 subfamily consists of two isoforms designated SULT2B1a and SULT2B1b (71). SULT2A1, which is the prototypical hydroxysteroid sulfotransferase, is commonly referred to as DHEA sulfotransferase because DHEA is considered the preferred substrate, although this isozyme has a broad substrate predilection (72, 73). In fact, the first human enzyme to be reported as exhibiting cholesterol sulfotransferase activity was SULT2A1, a conclusion that was based on biochemical and molecular studies involving liver samples (74). It was later determined, however, that the SULT2B1b isozyme is remarkably more active in sulfonating cholesterol than SULT2A1 (75). Interestingly, whereas the SULT2B1b isoform avidly sulfonates cholesterol, the SULT2B1a isoform preferentially sulfonates pregnenolone but not cholesterol (76).

From a structural point of view, the outstanding feature of the SULT2B1 isoforms, as compared with the SULT2A1 isozyme as well as other cloned steroid and cognate SULTs, is their extended amino- and carboxy-terminal ends (outlined in **Fig. 1** with dashed lines). All previously cloned members of the mammalian SULT superfamily, i.e., estrogen and phenol sulfotransferases as well as hydroxysteroid sulfotransferases, have sizes that range from 282 to 295 amino acids, whereas SULT2B1a and SULT2B1b consist of 350 and 365 amino acids, respectively. Overall, the SULT2A1 and SULT2B1 isozymes are ~37% identical at the amino acid level. If, however, the extended

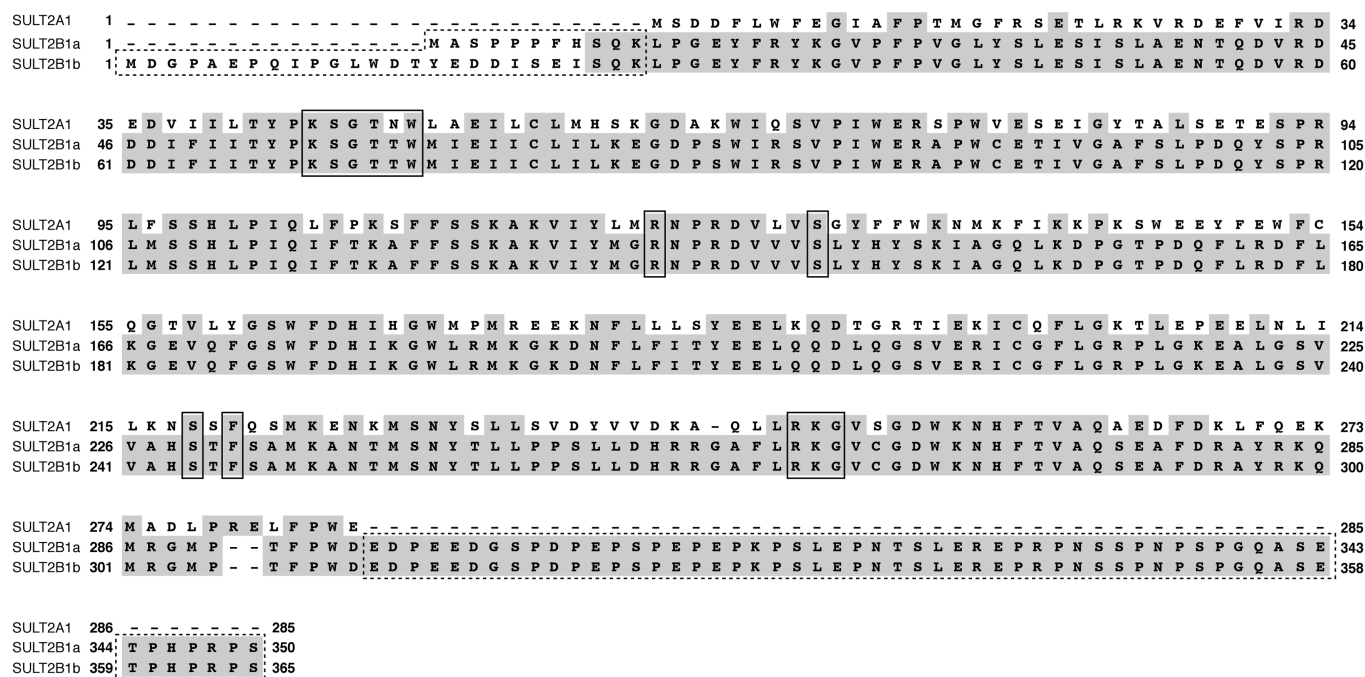


Fig. 1. Amino acid alignment of human cytosolic sulfotransferase 2A1 (SULT2A1), SULT2B1a, and SULT2B1b. Shading indicates identities. Dashed lines outline the extended amino- and carboxy-terminal ends of SULT2B1a and SULT2B1b. Boxed residues delineate conserved amino acids involved in 5'-phosphoadenosine-3'-phosphosulfate cofactor interaction (129, 130). Multiple alignment analysis was carried out using the MacVector 7.0 system, which is based on the Clustal W algorithm (131).

amino- and carboxy-terminal ends of the SULT2B1 isoforms are excluded, identities increase to $\sim 48\%$. Importantly, the extended amino- and carboxy-terminal ends of the SULT2B1 isoforms aside, there remains a significant structural similarity between the SULT2A1 and SULT2B1 isozymes in their core regions, which contain residues that are highly conserved in all SULTs; residues known to be involved in interaction with the 5'-phospho-adenosine-3'-phosphosulfate cofactor (these residues are enclosed in solid boxes in Fig. 1).

As illustrated in Fig. 2, human SULT2B1a and SULT2B1b are derived from a single gene (*SULT2B1*) as a result of an alternative exon I and differential splicing (71). Thus, the two SULT2B1 isoforms differ only at their N-terminal ends (cf. Fig. 1). Studies of the functional significance of this unique feature revealed that removal of 23 amino acids from the N-terminal end that is unique to SULT2B1b results in loss of cholesterol sulfotransferase activity; on the other hand, removal of eight amino acids from the N-terminal end that is unique to SULT2B1a has no effect on pregnenolone sulfotransferase activity (76). In the gene for *SULT2B1*, exon 1B encodes for only the unique N-terminal region of SULT2B1b, whereas exon 1A encodes for the unique N-terminal end of SULT2B1a plus an additional 48 amino acids (cf. Fig. 2). Thus, if the gene for *SULT2B1* employs exon 1B, cholesterol sulfotransferase is synthesized; however, if exon 1A is used, pregnenolone sulfotransferase is produced (76).

Studies regarding the functional significance of the extended carboxy-terminal ends of the SULT2B1 isoforms revealed that the terminal 53 amino acids can be removed from both isoforms without loss in catalytic activity (76). Nevertheless, the composition of the identical carboxy-terminal ends of the SULT2B1 isoforms is structurally interesting; this region is enriched in proline residues and contains proline, glutamic acid, serine, and threonine se-

quences (cf. Fig. 1) that may play a crucial role in targeting these isoforms for calpain proteolysis (77).

In keeping with the finding that cholesterol sulfate is widely present in human tissues, the mRNA for human SULT2B1b was also noted to be broadly expressed (75). Quantitatively, SULT2B1b mRNA is predominantly expressed in the skin, prostate, and placenta, and to a lesser extent in the stomach, small intestine, colon, kidney, lung, and thyroid gland (unpublished observations). It's of interest that skin, lungs, kidney, and intestines were also found to be major organs engaged in cholesterol sulfate synthesis in the guinea pig (78).

The human *SULT2B1* gene localizes to chromosome 19q13.3, ~ 500 kb telomeric to the location of the gene for *SULT2A1* (71). Although a substantial number of steroid sulfotransferase genes have now been cloned in several species, there has been little information forthcoming regarding their transcriptional regulation, with the exception of the *SULT2A1* gene in the rat; there are no reports as yet on the transcriptional regulation of the *SULT2B1* gene. Rat SULT2A1 is selectively manifest in liver, where expression is strongly repressed by androgens (79). In this regard, hepatocyte nuclear factor-1 and CCAAT/enhancer binding protein (C/EBP) response elements play pivotal roles (80). Concerning androgen repression, a negative androgen response region in the rat *SULT2A1* promoter has been mapped. Androgenic repression of the rat *SULT2A1* gene requires the presence of OCT-1 and C/EBP elements that map to specific promoter locations. Furthermore, the negative androgenic regulatory effect may be exerted indirectly through transcriptional interference of OCT-1 and C/EBP rather than via a direct interaction of the androgen receptor with DNA (80). In addition to its capacity as a neutral steroid sulfotransferase, rat SULT2A1 functions as bile acid sulfotransferase. Apropos to the latter function, rat SULT2A1 is inducible by pri-

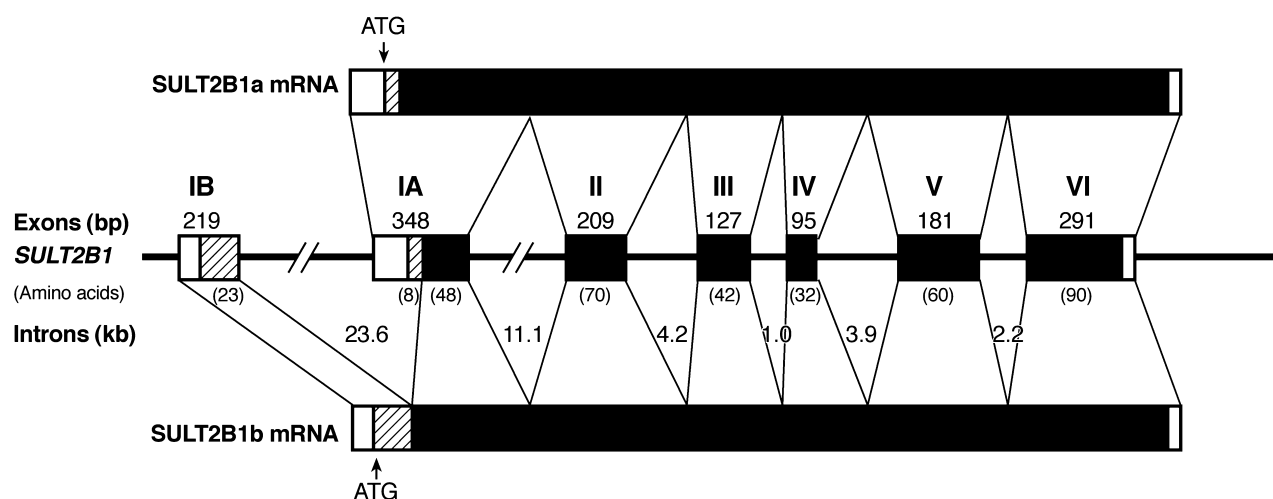


Fig. 2. Schema of the human *SULT2B1* gene demonstrating alternative exons IA and IB. The mRNAs for SULT2B1a and SULT2B1b are shown above and below, respectively, the linearized gene structure. Open boxes represent the 5' and 3' untranslated regions. The hatched boxes represent unique amino acid coding regions, whereas the solid boxes represent common amino acid coding regions. The DNA sequence of human *SULT2B1* was obtained from the human genome project and analyzed using cDNA of the SULT2B1 isoforms. A nucleotide alignment analysis, based on the Clustal W algorithm (131), was carried out using the MacVector 7.0 system.

mary bile acids in liver and intestinal cell lines, and this inducing effect is mediated through the bile acid-activated farnesoid X receptor (FXR), a member of the nuclear receptor super family (81). The ligand-activated FXR acts as a heterodimer with the 9-*cis*-retinoic acid receptor (RXR) and regulates the *SULT2A1* gene by binding to a *cis*-element cognate to the FXR-RXR- α heterodimer (81).

Hydrolysis of sulfonated steroids and sterols, which regenerates unconjugated substrates as free alcohols, completes the sulfurylation cycle and is carried out by a member of the sulfohydrolase or sulfatase gene family located on the X-chromosome at p22.3 (82). Steroid sulfatase (also referred to as arylsulfatase C) is a membrane-bound microsomal enzyme that is ubiquitously expressed in mammalian tissues and hydrolyzes a variety of 3 β -hydroxy-steroid sulfates, including cholesterol sulfate (83). The importance of cholesterol sulfate hydrolysis is exemplified by the genetic disorder X-linked ichthyosis, a disease characterized by severe scaling, levels of plasma cholesterol sulfate that are strikingly elevated (34, 35, 39), and excessive cholesterol sulfate deposition in the stratum corneum layer of the epidermis (45); furthermore, the increase of cholesterol sulfate levels in the stratum corneum, which results from steroid sulfatase deficiency, appears to be responsible for the ichthyotic changes (83). Little is known about how steroid sulfatase is regulated.

PHYSIOLOGIC SIGNIFICANCE

Steroid synthesis

Cholesterol sulfate can serve as a substrate for the synthesis of sulfonated adrenal steroids such as pregnenolone sulfate and DHEA sulfate without prior removal of the sulfate moiety (19–22). Although cholesterol sulfate is found in abundance in adrenal tissue, the human adrenal gland appears incapable of its synthesis (9, 78). Circulating cholesterol sulfate, at least in the rat, is not taken up by the adrenal gland (78). The latter finding is in keeping with the observation that human adrenal production of DHEA sulfate does not arise from cholesterol sulfate derived from the circulation (31), although a plausible explanation for this failure has been put forward (78). Thus, while the abundant amount of adrenal cholesterol sulfate would have to arise from either local synthesis and/or uptake from the circulation, its precise derivation remains to be definitively determined.

Evidence suggests that there are distinct adrenal mitochondrial side-chain cleavage (SCC) mixed function monooxygenase enzyme systems [cytochrome P₄₅₀ (P₄₅₀)] for converting cholesterol to pregnenolone and cholesterol sulfate to pregnenolone sulfate, although separation of these activities has not been attained (21, 84). It's notable that mitochondria isolated from human fetal adrenals have the ability to effectively convert large amounts of cholesterol sulfate to pregnenolone sulfate, suggesting that human fetal adrenal mitochondria contain a specific SCC P₄₅₀, and that cholesterol sulfate is the predominant

sterol substrate utilized in generating the large amounts of DHEA sulfate produced by this gland (85). On the other hand, mitochondria isolated from adult adrenals did not produce detectable amounts of pregnenolone sulfate from cholesterol sulfate (85).

Regarding gonadal utilization of cholesterol sulfate in steroidogenesis, mitochondria isolated from porcine and rodent ovaries have been noted to utilize cholesterol sulfate more efficiently as a substrate for SCC in the formation of pregnenolone sulfate than unconjugated or free cholesterol in the formation of pregnenolone (86). Similar studies, however, have not been carried out in humans.

Cholesterol sulfate's involvement in adrenal steroid synthesis may be two sided. That is, it can serve as a substrate for the mitochondrial SCC P₄₅₀ system, resulting in the formation of pregnenolone sulfate. Additionally, it has the ability to inhibit SCC of free or unconjugated cholesterol in the formation of pregnenolone (87). Interestingly, the locus of this inhibition is at the level of intramitochondrial cholesterol translocation and does not involve the SCC P₄₅₀ system itself (87). This observation has led to the suggestion that cholesterol sulfate might function as a physiologic regulator of cholesterol SCC activity and steroid synthesis (88).

Lipid metabolism

It has been shown that cholesterol sulfate added to cells in culture can have a significant influence on lipid metabolism. For example, when added to cultured skin fibroblasts, cholesterol sulfate inhibits sterol synthesis at the level of 3-hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting step in cholesterol synthesis (23). Since the levels of cholesterol sulfate are significantly higher in the epidermis than in other tissues, this finding has important implications for epidermal lipid metabolism (23). Whereas LDLs regulate cholesterol biosynthesis in most tissues, the epidermis does not possess this regulatory mechanism, although it is a major cholesterol-producing organ (89). In contrast to its inhibitory effect on sterogenesis in cell culture, cholesterol sulfate has a stimulatory influence on fatty acid synthesis (90). Additionally, as demonstrated *in vitro*, by inhibiting lecithin:cholesterol acyltransferase, cholesterol sulfate is capable of blocking the esterification of cholesterol (91).

Membranes

The finding that cholesterol sulfate is a normal constituent of red cell "ghosts" suggested that it might be involved in membrane structure and stability (33). This idea was supported by the finding that cholesterol sulfate at physiologic concentrations protected red blood cells against osmotic lysis, an effect requiring both the sulfate moiety and the cholesterol side chain (54, 92). This novel finding, along with the knowledge that cholesterol sulfate is widely distributed among human tissues, led to the hypothesis that cholesterol sulfate might be an important constituent of many types of biological membranes where it can function as a stabilizing agent (54). Soundness of the membrane stabilization concept was strengthened by

subsequent observations. For instance, Sendai virus-induced lysis of human erythrocytes was potently inhibited by cholesterol sulfate but not by cholesterol (93). For enveloped animal viruses such as the Sendai virus to enter cells first requires a fusion between the viral membrane and the cell plasma membrane, and inhibition of Sendai virus-induced hemolysis by cholesterol sulfate indicated there was a blockage in the fusion between the virus and red blood cell (93). Additional studies employing model membranes led to the proposal that cholesterol sulfate can alter the physical properties of membranes, which contributes to its ability to stabilize biological membranes and inhibit membrane fusion (93). Cholesterol sulfate interacts with phospholipids in a manner similar to that observed for cholesterol and has the ability to induce hydrocarbon ordering in lipid bilayers and to stabilize model membranes (94). Further studies with model membrane preparations revealed that the rate of intermembrane exchange for cholesterol sulfate is about an order of magnitude faster than that for cholesterol in all liposomes tested (95).

It has been proposed that hydrolysis of cholesterol sulfate within sperm membranes may lead to membrane destabilization and set into motion events that lead to capacitation (24, 96, 97). Cholesterol sulfate is present in relatively high concentrations in the male reproductive tract where it accumulates along the course of the epididymis and is avidly taken up and localized to the plasma membrane of spermatozoa, mostly to the acrosomal region. During sperm maturation in the epididymis, cholesterol sulfate acts as a membrane stabilizer and potent inhibitor of the protease acrosin (24, 97). Cleavage of the sulfate moiety within the female reproductive tract by a sterol sulfohydrolase reverses the inhibition, eliciting an acrosomal reaction that sets off a cascade of events leading to sperm capacitation and fertilization (24, 97).

Cholesterol sulfate has been shown to be a normal constituent of blood platelets and to modulate platelet function (52). For example, it is incorporated into platelet membranes where it potentiates ADP- and thrombin-induced platelet aggregation as well as serotonin secretion. These effects are specific for cholesterol sulfate and require both the sterol ring structure and the sulfate moiety (52). Furthermore, cholesterol sulfate modulates arachadonic acid metabolism while potentiating arachadonic acid-induced platelet aggregation; effects that are, in part, explained by changes in calcium flux (98). It has also been shown that platelets will adhere to cholesterol sulfate but not to cholesterol, other cholesterol esters, or other steroid sulfates under flow conditions similar to those seen in arteries (53).

Enzyme systems

Cholesterol sulfate can regulate the activity of a variety of functional proteins, some of which are activated. For instance, cholesterol sulfate has the ability to trigger the intrinsic blood coagulation system by activating Factor XII, an action that is not shared by other steroid sulfates or by unconjugated cholesterol (99). Furthermore, cholesterol sulfate activates prekallikrein in the presence of Factor

XII (99). Additionally, cholesterol sulfate activates multiple epidermal protein kinase C isozymes, especially the ϵ , η , and ζ isoforms (26). In vitro, cholesterol sulfate is a novel activator of the η isoform of protein kinase C, and in so doing is more potent than phosphatidylserine plus phorbol ester (27). The importance of these findings is that the η isoform of protein kinase C is predominantly expressed in the suprabasal region of the human epidermis, especially by cells in the granular layer, where it is involved in barrier formation (100). Finally, high-mobility group protein 1 (HMG1), a component of chromatin involved in the modulation of transcription, is phosphorylated on specific threonine residues by casein kinase I when incubated with cholesterol sulfate, whereas no phosphorylation of HMG1 was detected in the presence of other cholesterol-related compounds or their sulfated derivatives (101). This result suggests that cholesterol sulfate may singly function to induce the phosphorylation of HMG1 by casein kinase I (101). A marked change in the conformation of HMG1 occurs in the presence of cholesterol sulfate, suggesting a mechanism to explain how this sterol sulfate alone can induce the phosphorylation of HMG1 by casein kinase I (101).

In contrast to the above stimulatory effects of cholesterol sulfate, other functional proteins are inhibited by this sulfolipid. For example, thrombin and plasmin, serine proteases that play essential roles in blood clotting and fibrinolysis, respectively, are potently inhibited by cholesterol sulfate (25). It is suggested that cholesterol sulfate serves as an alternative physiologic inhibitor of thrombin in human plasma (25). Additionally, cholesterol sulfate suppresses sterol synthesis in cultured human fibroblasts by acting as a robust inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, suggesting that cholesterol sulfate may be an endogenous regulator of cholesterol synthesis (23). Interestingly, cholesterol sulfate can modulate the specificity of phosphatidylinositol-3-kinase. That is, at low micromolar concentrations, in contrast to unconjugated cholesterol or the sulfoconjugated steroid, androsterone cholesterol sulfate selectively inhibits phosphatidylinositol-3-kinase activity, whereas it has a negligible effect on the activity of phosphatidylinositol bisphosphate 3-kinase (102). This effect is likely mediated by the catalytic subunit p100a, indicating that this subunit could have a binding site for cholesterol sulfate (102). This in vitro result has interesting physiologic implications regarding the distinctive in vivo substrate specificity demonstrated by phosphatidylinositol-3-kinase.

Cholesterol sulfate has been noted to inhibit the pancreatic serine proteases trypsin and chymotrypsin (103, 104) as well as a yeast pronase (103); it also inhibits pancreatic elastase (105) and DNase I (106). The physiologic significance of these in vitro findings, however, is not fully appreciated. Nonetheless, one pathologic implication has been considered since normal desquamation of the stratum corneum involves trypsin-like and chymotrypsin-like proteases (107, 108). The trypsin- and chymotrypsin-inhibitory property demonstrated by cholesterol sulfate in

vitro has led to the postulate that an excess of this sulfolipid might affect the breakdown of desmosomes normally required for desquamation (104). Thus, in the disease known as recessive X-linked ichthyosis, this mechanism might be responsible for the retention hyperkeratosis, abnormal scaling, and defective skin barrier characteristic of this disorder (104), which, as noted previously, is a condition associated with abnormally high cholesterol sulfate levels in the stratum corneum resulting from steroid sulfatase deficiency (35, 45, 109).

CHOLESTEROL SULFATE AS A REGULATORY MOLECULE IN SKIN

During skin development, modifications involving the outer layer of the epidermis result in the creation of an effective physical barrier of cornified squamous cells termed the stratum corneum (110). The bulwark of this epidermal barrier is composed of a layer of flattened anucleated corneocytes mortared together by lipid lamellae atop a layer of cross-linked cell envelope proteins anchored to a keratin filament-matrix complex (69). Structural defects in any of the components of the cornified squamous epithelial covering can cause clinically significant disease as a result of impairment in barrier function (69).

Substantial evidence has accumulated demonstrating an important role for cholesterol sulfate in keratinocyte differentiation and development of the barrier (26, 111–113). Cholesterol sulfate formation occurs early on in human squamous development, suggesting a functional role at an early stage in differentiation (28). Cholesterol sulfate is rich in the upper layers of the human epidermis, where it gradually accumulates during keratinocyte differentiation, reaching levels that are 4–5% of total lipid content in the upper stratum granulosum (58, 59), after which it undergoes sulfohydrolysis in the cornified layer and representation drops to 1% of total lipids (114, 115). The cholesterol-cholesterol sulfate ratio in epithelial cells outside of the skin is normally about 500:1 (116), whereas in the normal stratum corneum the ratio is as low as 10:1 or 5:1 (117). In the recessive X-linked ichthyosis disorder, however, which is characterized by a deficiency in steroid sulfatase activity (109), the cholesterol-cholesterol sulfate ratio in the stratum corneum is 1:1 (45).

Consistent with the involvement of cholesterol sulfate in keratinocyte differentiation and barrier development is the predominant expression by quantitative real-time PCR of SULT2B1b mRNA in human skin (unpublished observations). In contrast, expression of SULT2A1 appears not to occur in human skin (75). The selective expression of SULT2B1b in skin also occurs in other species, such as the mouse (118). In cultured epidermal cells, calcium induces terminal differentiation (119, 120). Normal human keratinocytes (NHKs) maintained in primary culture progressively express SULT2B1b mRNA and protein during terminal differentiation in response to calcium (illustrated in Fig. 3). Involucrin, a major cross-linked protein component of the cornified cell envelope, is an

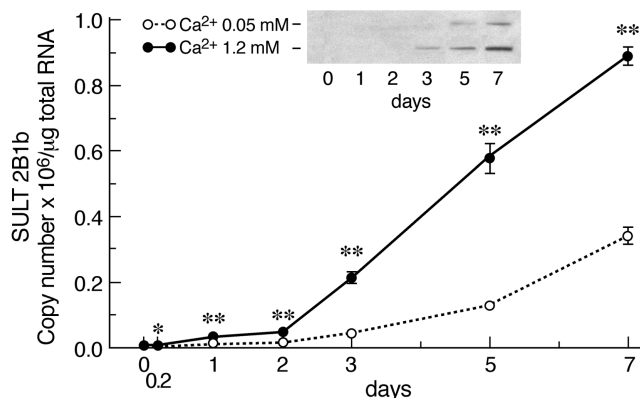


Fig. 3. Expression of SULT2B1b by normal human keratinocytes (NHKs) in primary culture at two calcium concentrations. SULT2B1b mRNA was quantified by real-time PCR and expressed as copy number per total RNA. SULT2B1b protein expression by Western analysis is indicated in the inset. Asterisks indicate significance.

early marker of keratinocyte differentiation (121, 122). Interestingly, as shown in Fig. 4, cholesterol sulfate will induce expression of involucrin mRNA when added to cultured NHK.

Cholesterol sulfate is thought to play a significant role in the proper functioning of the stratum corneum and the normal sloughing of dead cells, i.e., the process of desquamation (114). It has been shown that cholesterol sulfate retards desquamation by inhibiting serine proteases in the stratum corneum that are responsible for desmosomal degradation (104). In addition to desmosomes, which form junctions between adjacent cells and are involved in adhesion of corneocytes, lipid bilayers play a role in intercellular cohesion in the stratum corneum, and a decrease in cholesterol sulfate content can affect the stratum corneum lipid phase behavior (113). A reduction in cholesterol sulfate content in the stratum corneum decreases the stability of bilayers and reduces the fraction of lipids arranged in liquid lateral packing, which results in a reduction in the elasticity of the lipid phases, preventing proper corneocyte lipid lamellae orientation (113, 123).

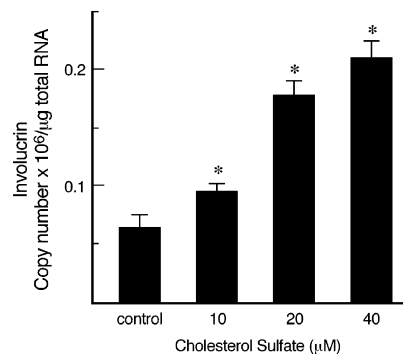


Fig. 4. Cholesterol sulfate induction of involucrin mRNA expression in NHKs in primary culture. Involucrin mRNA was quantified by real-time PCR and expressed as copy number per total RNA. Tops of columns represent mean values, and error bars indicate standard deviations. Asterisks denote significance.

The end result is a decrease in cohesion between cells and the promotion of cell shedding or desquamation. Relatedly, the horse hoof is particularly rich in cholesterol sulfate, which significantly contributes to the high degree of cohesiveness of this fully keratinized tissue (124).


Cholesterol sulfate induces transcription of the gene for transglutaminase I, an essential protein cross-linking enzyme involved in formation of the barrier (29). Furthermore, it is suggested that cholesterol sulfate stimulation of the transglutaminase I gene is secondary to activation of the η isoform of protein kinase C (29), an isoform expressed in cells of the granular layer of the epidermis (100). Cholesterol sulfate, by functioning as a transcriptional regulator, increases the mRNA for involucrin (125). As noted previously, involucrin is a major cross-linked protein constituent of the insoluble cornified cell envelope that is expressed during the early stages of terminal keratinocyte differentiation (121, 122). The cholesterol sulfate-responsive region of the involucrin gene contains an AP-1 site, whereby cholesterol sulfate treatment leads to an increase in AP-1-DNA binding; furthermore, both mRNA and protein levels of several members of the AP-1 class of transcription factors, which includes the *Jun* and *Fos* family proteins (126), are increased by this sulfolipid (125). For instance, Fra-1, Fra-2, and Jun D, components of the AP-1-DNA-binding complex, are increased by cholesterol sulfate. These novel findings suggest that cholesterol sulfate regulates early stages of terminal keratinocyte differentiation by adjusting levels of specific AP-1 proteins, which in turn regulate expression of the genes involved in this process, such as the involucrin gene (125).

Although fetal developmental studies are not available for humans, studies of cholesterol sulfotransferase and steroid sulfatase enzymatic activities in the rat epidermis during fetal development revealed that cholesterol sulfotransferase activity increases ~10-fold between Day 17 and Day 19, after which it declines between Day 19 and Day 21 (127). Steroid sulfatase activity, on the other hand, peaks on Day 21 after increasing more than 5-fold (127). These results, plus additional studies, suggested that the cholesterol sulfate synthetic and degradative enzyme systems are differentially regulated and that their induction during ontogenesis of the stratum corneum is a component of the fetal epidermal differentiation program (127).

CONCLUDING REMARKS

The sulfate moiety of cholesterol sulfate has a pK_a of ~3.3, indicating that it is normally ionized under physiologic conditions (54). Thus, the sulfoconjugation of cholesterol results in the conversion of a rather rigid hydrophobic molecule into a very amphiphilic compound containing a highly charged subgroup. The hydrophobic/hydrophilic property of cholesterol sulfate undoubtedly represents an important characteristic underlying its biologic action. This aspect of the structure of cholesterol sulfate is thought to make it ideally suited for interactions with membrane constituents (54). Indeed, much of the

early research on the physiologic relevance of cholesterol sulfate was focused in the area of binding to membrane components with enhanced membrane stabilization. More recently, however, has been the exciting development whereby cholesterol sulfate has demonstrated the ability to function as a regulatory molecule. In this capacity, it has been shown to influence cell signaling by activating the protein kinase C pathway via interaction with specific C-kinase isoforms (26, 100). Furthermore, either via the C-kinase pathway or by another as yet unknown mechanism that modulates the activity AP-1 transcription factors, cholesterol sulfate has demonstrated a regulatory influence on the transcriptional activity of specific keratinocyte genes (29, 125). Our ability to understand the physiologic significance of this intriguing molecule is greatly augmented by the cloning of the gene for the sulfotransferase (SULT2B1b) that sulfonates cholesterol (71). Thus, definitive information on the transcriptional regulation of the human *SULT2B1* gene, as well as regulation of specific tissue expression of the SULT2B1b isoform should be forthcoming in the near future. Furthermore, cloning of the mouse ortholog of the *SULT2B1* gene is an important achievement that will initiate studies pertaining to embryogenesis and specific tissue expression during growth and development (118). While cholesterol sulfate has been shown to induce the transcription of important genes involved in keratinocyte development, it is not clear that this is necessarily its only function or even a major function. Certainly, the role of cholesterol sulfate in barrier formation and epidermal cell adhesion needs to be further explored. It would be of interest to examine the involvement of cholesterol sulfate in protein-protein interactions, such as between transglutaminase 3 and protein substrates in the upper granular layer of the epidermis (128). In addition to human skin (75), the enzyme producing cholesterol sulfate (SULT2B1b) is also highly expressed in the human prostate and placenta (71, 75); however, the basis for the high expression in these tissues is not understood and needs to be explored.

Cholesterol sulfate has emerged as a significant lipid constituent in a variety of human tissues, although the definitive biologic import of the presence of this sulfolipid in any specific tissue continues to evolve. The fact that cholesterol sulfate circulates in blood in combination with its emerging role as a regulatory molecule suggests a similarity to compounds categorized as hormones. 

REFERENCES

1. Huxtable, R. J. 1986. Biochemistry of Sulfur. Plenum Publishing Corp., New York.
2. Strott, C. A. 2002. Sulfonation and molecular action. *Endocr. Rev.* **23**: 703-732.
3. Mulder, G. J., and W. B. Jakoby. 1990. Sulfation. In *Conjugation Reactions in Drug Metabolism: An Integrated Approach*. G. J. Mulder, editor. Taylor & Francis, New York. 107-161.
4. Makita, A., and N. Taniguchi. 1985. Sulfoglycolipids. In *New Comprehensive Biochemistry*. H. Wiegandt, editor. Elsevier, Amsterdam. 1-99.
5. Vos, J. P., M. Lopes-Cardozo, and B. M. Gadella. 1994. Metabolic

- and functional aspects of sulfogalactolipids. *Biochim. Biophys. Acta*. **1211**: 125–149.
6. Kuchel, O., N. T. Buu, and O. Serri. 1982. Sulfoconjugation of catecholamines, nutrition, and hypertension. *Hypertension*. **4**: III-93–III-98.
 7. Visser, T. J. 1994. Role of sulfation in thyroid hormone metabolism. *Chem. Biol. Interact.* **92**: 293–303.
 8. Tolbert, B. M. 1985. Metabolism and function of ascorbic acid and its metabolites. *Int. J. Vit. Nutr. Res.* **27 (Suppl.)**: 121–138.
 9. Roberts, K. D., and S. Lieberman. 1970. The biochemistry of the 3 β -hydroxy- Δ^2 -steroid sulfates. In *Chemical and Biological Aspects of Steroid Conjugation*. S. Bernstein and S. Solomon, editors. Springer-Verlag, New York. 219–290.
 10. Palmer, R. H., and M. G. Bolt. 1971. Synthesis of lithocholic acid sulfates and their identification in human bile. *J. Lipid Res.* **12**: 671–679.
 11. Nagubandi, S., J. M. Lonndowski, S. Bollman, P. Tietz, and R. Kumar. 1981. Synthesis and biological activity of vitamin D₃ 3 β -sulfate. *J. Biol. Chem.* **256**: 5536–5539.
 12. Axelson, M. 1985. 25-hydroxyvitamin D₃ 3-sulfate is a major circulating form of vitamin D in man. *FEBS Lett.* **191**: 171–175.
 13. Roy, A. B., and P. Trudinger. 1970. The Biochemistry of Inorganic Compounds of Sulphur. Cambridge University Press, London.
 14. Siiteri, P. K. 1970. The isolation of steroid conjugates. In *Chemical and Biological Aspects of Steroid Conjugation*. S. Bernstein and S. Solomon, editors. Springer-Verlag, New York. 182–218.
 15. Drayer, N. M., K. D. Roberts, L. Bandi, and S. Lieberman. 1964. The isolation of cholesterol sulfate from bovine adrenals. *J. Biol. Chem.* **239**: 3112–3114.
 16. Drayer, N. M., and S. Lieberman. 1965. Isolation of cholesterol sulfate from human blood and gallstones. *Biochem. Biophys. Res. Commun.* **18**: 126–130.
 17. Moser, H. W., A. B. Moser, and J. C. Orr. 1966. Preliminary observations on the occurrence of cholesterol sulfate in man. *Biochim. Biophys. Acta*. **116**: 146–155.
 18. Drayer, N. M., and S. Lieberman. 1967. Isolation of cholesterol sulfate from human aortas and adrenal tumors. *J. Clin. Endocrinol. Metab.* **27**: 136–139.
 19. Roberts, K. D., L. Bandi, H. I. Calvin, W. D. Drucker, and S. Lieberman. 1964. Evidence that steroid sulfates serve as biosynthetic intermediates. IV. Conversion of cholesterol sulfate *in vivo* to urinary C₁₉ and C₂₁ steroidal sulfates. *Biochemistry*. **3**: 1983–1988.
 20. Roberts, K. D., L. Bandy, and S. Lieberman. 1967. The conversion of cholesterol-³H-sulfate-³⁵S into pregnenolone-³H-sulfate-³⁵S by sonicated bovine adrenal mitochondria. *Biochem. Biophys. Res. Commun.* **29**: 741–746.
 21. Young, D. G., and P. F. Hall. 1969. The side-chain cleavage of cholesterol and cholesterol sulfate by enzymes from bovine adrenocortical mitochondria. *Biochemistry*. **8**: 2987–2996.
 22. Hochberg, R. B., S. Ladany, M. Welch, and S. Lieberman. 1974. Cholesterol and cholesterol sulfate as substrates for the adrenal side-chain cleavage enzyme. *Biochemistry*. **13**: 1938–1945.
 23. Williams, M. L., M. Hughes-Fulford, and P. M. Elias. 1985. Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and sterol synthesis by cholesterol sulfate in cultured fibroblasts. *Biochim. Biophys. Acta*. **845**: 349–357.
 24. Langlais, J., M. Zollinger, L. Plante, A. Chapdelaine, G. Bleau, and K. D. Roberts. 1981. Localization of cholesteryl sulfate in human spermatozoa in support of a hypothesis for the mechanism of capacitation. *Proc. Natl. Acad. Sci. USA*. **78**: 7266–7270.
 25. Iwamori, M., Y. Iwamori, and N. Ito. 1999. Regulation of the activities of thrombin and plasmin by cholesterol sulfate as a physiological inhibitor in human plasma. *J. Biol. Chem.* **274**: 594–601.
 26. Denning, M. F., M. G. Kazanietz, P. M. Blumberg, and S. H. Yuspa. 1995. Cholesterol sulfate activates multiple protein kinase C isozymes and induces granular cell differentiation in cultured murine keratinocytes. *Cell Growth Differ.* **6**: 1619–1626.
 27. Ikuta, T., K. Chida, O. Tajima, Y. Matsuura, M. Iwamori, Y. Ueda, K. Mizuno, S. Ohno, and T. Kuroki. 1994. Cholesterol sulfate, a novel activator for the ϵ isoform of protein kinase C. *Cell Growth Differ.* **5**: 943–947.
 28. Jetten, A. M., M. A. George, C. Nervi, L. R. Boone, and J. I. Rearick. 1989. Increased cholesterol sulfate and cholesterol sulfotransferase activity in relation to the multi-step process of differentiation in human epidermal keratinocytes. *J. Invest. Dermatol.* **92**: 203–209.
 29. Kawabe, S., T. Ikuta, M. Ohba, K. Chida, Y. Ueda, K. Yamanishi, and T. Kuroki. 1998. Cholesterol sulfate activates transcription of transglutaminase 1 gene in normal human keratinocytes. *J. Invest. Dermatol.* **111**: 1098–1102.
 30. Paul, S. M., and R. H. Purdy. 1992. Neuroactive steroids. *FASEB J.* **6**: 2311–2322.
 31. Gurrpide, E., K. D. Roberts, M. T. Welch, L. Bandy, and S. Lieberman. 1966. Studies on the metabolism of blood-borne cholesterol sulfate. *Biochemistry*. **5**: 3352–3362.
 32. Winter, J. S. D., and A. M. Bongiovanni. 1968. Identification of cholesterol sulfate in urine and plasma of normal and hypercholesterolemic subjects. *J. Clin. Endocrinol. Metab.* **28**: 927–930.
 33. Bleau, G., A. Chapdelaine, and K. D. Roberts. 1972. The assay of cholesterol sulfate in biological material by enzymatic radioisotopic displacement. *Can. J. Biochem.* **50**: 277–286.
 34. Bergner, E. A., and L. J. Shapiro. 1981. Increased cholesterol sulfate in plasma and red blood cell membranes of steroid sulfatase deficient patients. *J. Clin. Endocrinol. Metab.* **53**: 221–223.
 35. Epstein, E. H., R. M. Krauss, and C. Shackleton. 1981. X-linked ichthyosis: increased blood cholesterol sulfate and electrophoretic mobility of low-density lipoprotein. *Science*. **214**: 659–660.
 36. Huang, Y. S., K. Eid, and J. Davignon. 1981. Cholesteryl sulfate: measurement with β -sitosteryl sulfate as an internal standard. *Can. J. Biochem.* **59**: 602–605.
 37. Muskiet, F. A., G. Jansen, B. G. Wolthers, A. Marinkovic-Ilsen, and P. C. van Voorst Vader. 1983. Gas-chromatographic determination of cholesterol sulfate in plasma and erythrocytes, for the diagnosis of recessive X-linked ichthyosis. *Clin. Chem.* **29**: 1404–1407.
 38. Serizawa, S., T. Nagai, and Y. Sato. 1987. Simplified method of determination of serum cholesterol sulfate by reverse phase thin-layer chromatography. *J. Invest. Dermatol.* **89**: 580–587.
 39. Serizawa, S., T. Nagai, and Y. Sato. 1989. Simplified determination of serum cholesterol sulfate by gas-liquid chromatography combined with cyclohexylsilane-bonded phase column purification. *Arch. Dermatol. Res.* **281**: 411–416.
 40. Veares, M. P., R. P. Evershed, M. C. Prescott, and L. J. Goad. 1990. Quantitative determination of cholesterol sulfate in plasma by stable isotope dilution fast atom bombardment mass spectrometry. *Biomed. Environ. Mass Spectrom.* **19**: 583–588.
 41. Tamasawa, N., A. Tamasawa, and K. Takebe. 1993. Higher levels of plasma cholesterol sulfate in patients with liver cirrhosis and hypercholesterolemia. *Lipids*. **28**: 833–836.
 42. Nakamura, T., Y. Matsuzawa, M. Okano, Y. Kitano, T. Funahashi, S. Yamashita, and S. Tarui. 1988. Characterization of low-density lipoproteins from patients with recessive ichthyosis. *Atherosclerosis*. **70**: 43–52.
 43. Blache, D., C. Rodriguez, and J. Davignon. 1995. Enhanced susceptibility of cholesteryl sulfate-enriched low density lipoproteins to copper-mediated oxidation. *FEBS Lett.* **362**: 197–200.
 44. van Doormaal, J. J., F. A. Muskiet, G. Jansen, B. G. Wolthers, W. J. Sluiter, and H. Doorenbos. 1986. Increase of plasma and red cell cholesterol sulfate levels in induced hypothyroidism in man. *Clin. Chim. Acta*. **155**: 195–200.
 45. Williams, M. L., and P. M. Elias. 1981. Stratum corneum lipids in disorders of cornification. *J. Clin. Invest.* **68**: 1404–1410.
 46. Hopper, B. R., and S. S. C. Yen. 1975. Circulating concentrations of dehydroepiandrosterone and dehydroepiandrosterone sulfate during puberty. *J. Clin. Endocrinol. Metab.* **40**: 458–461.
 47. Orentreich, N., J. L. Brind, R. L. Rizer, and J. H. Vogelmann. 1984. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J. Clin. Endocrinol. Metab.* **59**: 551–555.
 48. Lin, B., K. Kubushiro, Y. Akiba, Y. Cui, K. Tsukazaki, S. Nozawa, and M. Iwamori. 1997. Alteration of acidic lipids in human sera during the course of pregnancy: characteristic increase in the concentration of cholesterol sulfate. *J. Chromatogr. B*. **704**: 99–104.
 49. Eneroth, P., and E. Nystrom. 1968. Quantification of cholesterol sulfate and neutral sterol derivatives in human feces after purification on lipophilic sephadex gels. *Steroids*. **11**: 187–204.
 50. Eneroth, P., and E. Nystrom. 1968. Sterol composition of the sterol sulfate fraction in human feces. *Steroids*. **11**: 417–427.
 51. Sion, B., G. Grizard, and D. Boucher. 2001. Quantitative analysis of desmosterol, cholesterol and cholesterol sulfate in semen by high-performance liquid chromatography. *J. Chromatogr. A*. **935**: 259–265.
 52. Blache, D., M. Becchi, and J. Davignon. 1995. Occurrence and bi-

- ological effects of cholesteryl sulfate on blood platelets. *Biochim. Biophys. Acta*. **1259**: 291–296.
53. Merten, M., J. F. Dong, J. A. Lopez, and P. Thiagarajan. 2001. Cholesterol sulfate. A new adhesive molecule for platelets. *Circulation*. **103**: 2032–2034.
 54. Bleau, G., F. H. Bodley, J. Longpre, A. Chapdelaine, and K. D. Roberts. 1974. Cholesterol sulfate I. Occurrence and possible function as an amphipathic lipid in the membrane of the human erythrocyte. *Biochim. Biophys. Acta*. **352**: 1–9.
 55. Iwamori, M., H. W. Moser, and Y. Kishimoto. 1976. Cholesterol sulfate in rat tissues. *Biochim. Biophys. Acta*. **441**: 268–279.
 56. Gray, G. M., and H. J. Yardley. 1975. Lipid compositions of cells isolated from pig, human, and rat epidermis. *J. Lipid Res.* **16**: 434–440.
 57. Epstein, E. H., Jr., M. L. Williams, and P. M. Elias. 1984. The epidermal cholesterol sulfate cycle. *J. Am. Acad. Dermatol.* **10**: 866–868.
 58. Elias, P. M., M. L. Williams, M. E. Maloney, J. A. Bonifas, B. E. Brown, S. Grayson, and E. H. Epstein, Jr. 1984. Stratum corneum lipids in disorders of cornification. *J. Clin. Invest.* **74**: 1414–1421.
 59. Lampe, M. A., M. L. Williams, and E. R. Elias. 1983. Human epidermal lipids: characterization and modulations during differentiation. *J. Lipid Res.* **24**: 131–140.
 60. Ponc, M., and M. L. Williams. 1986. Cholesterol sulfate uptake and outflux in cultured human keratinocytes. *Arch. Dermatol. Res.* **279**: 32–36.
 61. Wertz, P. W., and D. T. Downing. 1988. Integral lipids of human hair. *Lipids*. **23**: 878–881.
 62. Serizawa, S., T. Nagai, M. Ito, and Y. Sato. 1990. Cholesterol sulphate levels in the hair and nails of patients with recessive X-linked ichthyosis. *Clin. Exp. Dermatol.* **15**: 13–15.
 63. Longcope, C. 1995. Metabolism of dehydroepiandrosterone. *Ann. N. Y. Acad. Sci.* **774**: 143–148.
 64. Wang, D. Y., and R. D. Bulbrook. 1967. The metabolic clearance rates of dehydroepiandrosterone, testosterone and their sulphate esters in man, rat and rabbit. *J. Endocrinol.* **38**: 307–318.
 65. Plager, J. E. 1965. The binding of androsterone sulfate, etiocholanolone sulfate, and dehydroisoandrosterone sulfate by human plasma protein. *J. Clin. Invest.* **44**: 1234–1239.
 66. Baulieu, E.-E., C. Corpechot, F. Dray, R. Emiliozzi, M.-C. Lebeau, P. Mauvais-Jarvis, and P. Robel. 1965. An adrenal-secreted androgen: dehydroepiandrosterone sulfate. Its metabolism and a tentative generalization on the metabolism of other steroid conjugates in man. *Recent Prog. Horm. Res.* **21**: 411–500.
 67. Vande Wiele, R. L., P. C. MacDonald, E. Gurrpide, and S. Lieberman. 1963. Studies on the secretion and interconversion of the androgens. *Recent Prog. Horm. Res.* **19**: 275–290.
 68. Endoh, A., S. B. Kristiansen, P. R. Casson, J. E. Buster, and P. J. Hornsby. 1996. The zona reticularis is the site of biosynthesis of dehydroepiandrosterone and dehydroepiandrosterone sulfate in the adult adrenal cortex resulting from its low expression of 3 β -hydroxysteroid dehydrogenase. *J. Clin. Endocrinol. Metab.* **81**: 3558–3565.
 69. Roop, D. 1995. Defects in the barrier. *Science*. **267**: 474–475.
 70. Nagata, K., and Y. Yamazoe. 2000. Pharmacogenetics of sulfotransferase. *Annu. Rev. Pharmacol. Toxicol.* **40**: 159–176.
 71. Her, C., T. C. Wood, E. E. Eichler, H. W. Mohrenweiser, L. S. Ramagli, M. J. Siciliano, and R. M. Weinshilboum. 1998. Human hydroxysteroid sulfotransferase SULT2B1: two enzymes encoded by a single chromosome 19 gene. *Genomics*. **53**: 284–295.
 72. Falany, C. N., M. E. Vazquez, and J. M. Kalb. 1989. Purification and characterization of human dehydroepiandrosterone sulfotransferase. *Biochem. J.* **260**: 641–646.
 73. Hernandez, J. S., R. W. G. Watson, T. C. Wood, and R. M. Weinshilboum. 1992. Sulfation of estrone and 17 β -estradiol in human liver: catalysis by thermostable phenol sulfotransferase and by dehydroepiandrosterone sulfotransferase. *Drug Metab. Dispos.* **20**: 413–422.
 74. Askoy, A., D. M. Otterness, and R. M. Weinshilboum. 1993. Cholesterol sulfation in human liver: catalysis by dehydroepiandrosterone sulfotransferase. *Drug Metab. Dispos.* **21**: 268–276.
 75. Javitt, N. B., Y. C. Lee, C. Shimizu, H. Fuda, and C. A. Strott. 2001. Cholesterol and hydroxycholesterol sulfotransferases: identification, distinction from dehydroepiandrosterone sulfotransferase, and differential tissue expression. *Endocrinology*. **142**: 2978–2984.
 76. Fuda, H., Y. C. Lee, C. Shimizu, N. B. Javitt, and C. A. Strott. 2002. Mutational analysis of human hydroxysteroid sulfotransferase SULT2B1 isoforms reveals that exon 1B of the *SULT2B1* gene produces cholesterol sulfotransferase, whereas exon 1A yields pregnenolone sulfotransferase. *J. Biol. Chem.* **277**: 36161–36166.
 77. Rechsteiner, M., and S. W. Rogers. 1996. PEST sequences and regulation by proteolysis. *Trends Biochem. Sci.* **21**: 267–271.
 78. Hochberg, R. B., S. Ladany, and S. Lieberman. 1974. Cholesterol sulfate: some aspects of its biosynthesis and uptake by tissues from blood. *Endocrinology*. **94**: 207–213.
 79. Demian, W. F., C. S. Song, D. S. Kim, S. Her, W. Gallwitz, T. R. Rao, M. Slomczynska, B. Chatterjee, and A. K. Roy. 1992. Estrogen sulfotransferase of the rat liver: complementary DNA cloning and age- and sex-specific regulation of messenger RNA. *Mol. Endocrinol.* **6**: 589–597.
 80. Song, C. S., M. H. Jung, S. C. Kim, T. Hassan, A. K. Roy, and B. Chatterjee. 1998. Tissue-specific and androgen-repressible regulation of the rat dehydroepiandrosterone sulfotransferase gene promoter. *J. Biol. Chem.* **273**: 21856–21866.
 81. Song, C. S., I. Echchgadda, B.-S. Baek, S. C. Ahn, A. K. Roy, and B. Chatterjee. 2001. Dehydroepiandrosterone sulfotransferase gene induction by bile acid activated farnesoid X receptor. *J. Biol. Chem.* **276**: 42549–42556.
 82. Parenti, G., G. Meroni, and A. Ballabio. 1997. The sulfatase gene family. *Curr. Opin. Genet. Dev.* **7**: 386–391.
 83. Ballabio, A., and L. J. Shapiro. 1995. Steroid sulfatase deficiency and X-linked ichthyosis. In *The Metabolic and Molecular Bases of Inherited Disease*. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, Inc., New York. 2999–3022.
 84. Wolfson, A., and S. Lieberman. 1979. Evidence suggesting that more than one sterol side chain cleavage enzyme system exists in mitochondria from bovine adrenal cortex. *J. Biol. Chem.* **254**: 4096–4100.
 85. Mason, J. I., and P. G. Hemsell. 1982. Cholesterol sulfate metabolism in human fetal adrenal mitochondria. *Endocrinology*. **111**: 208–213.
 86. Tuckey, R. C. 1990. Side-chain cleavage of cholesterol sulfate by ovarian mitochondria. *J. Steroid Biochem. Mol. Biol.* **37**: 121–127.
 87. Lambeth, J. D., X. X. Xu, and M. Glover. 1987. Cholesterol sulfate inhibits adrenal mitochondrial cholesterol side-chain cleavage at a site distinct from cytochrome P-450_{SCC}. *J. Biol. Chem.* **262**: 9181–9188.
 88. Lambeth, J. D., and X. X. Xu. 1989. Cholesterol sulfate: a naturally-occurring inhibitor of cholesterol side-chain cleavage which functions at the level of intramitochondrial cholesterol translocation. *Endocr. Res.* **15**: 85–99.
 89. Ponc, M., L. Havekes, J. Kempenaar, and B. Vermeer. 1984. Cultured human skin fibroblasts and keratinocytes: differences in the regulation of cholesterol synthesis. *J. Invest. Dermatol.* **81**: 125–130.
 90. Williams, M. L., S. L. Rutherford, and K. R. Feingold. 1987. Effects of cholesterol sulfate on lipid metabolism in cultured human keratinocytes and fibroblasts. *J. Lipid Res.* **28**: 955–967.
 91. Nakagawa, M., and S. Kojima. 1976. Effect of cholesterol sulfate and sodium dodecyl sulfate on lecithin-cholesterol acyltransferase in human plasma. *J. Biochem.* **80**: 729–733.
 92. Bleau, G., G. Lalumiere, A. Chapdelaine, and K. D. Roberts. 1975. Red cell surface structure. Stabilization by cholesterol sulfate as evidenced by electron microscopy. *Biochim. Biophys. Acta*. **375**: 220–223.
 93. Cheetham, J. J., R. M. Epand, M. Andrews, and T. D. Flanagan. 1990. Cholesterol sulfate inhibits the fusion of Sendai virus to biological and model membranes. *J. Biol. Chem.* **265**: 12404–12409.
 94. Kitson, N., M. Monck, K. Wong, J. Thewalt, and P. Cullis. 1992. The influence of cholesterol 3-sulphate on phase behavior and hydrocarbon order in model membrane systems. *Biochim. Biophys. Acta*. **1111**: 127–133.
 95. Rodriguez, W. V., J. J. Wheeler, S. K. Klimuk, C. N. Kitson, and M. J. Hope. 1995. Transbilayer movement and net flux of cholesterol and cholesterol sulfate between liposomal membranes. *Biochemistry*. **34**: 6208–6217.
 96. Lalumiere, G., G. Bleau, A. Chapdelaine, and K. D. Roberts. 1976. Cholesteryl sulfate and sterol sulfatase in the human reproductive tract. *Steroids*. **27**: 247–260.
 97. Roberts, K. D. 1987. Sterol sulfates in the epididymis: synthesis and possible function in the reproductive process. *J. Steroid Biochem.* **27**: 337–341.
 98. Blache, D. 1996. Enhanced arachidonic acid and calcium metabolism in cholesteryl sulfate-enriched rat platelets. *J. Lipid Mediat. Cell Signal.* **13**: 127–138.

99. Shimada, T., H. Kato, S. Iwanaga, M. Iwamori, and Y. Nagai. 1985. Activation of factor XII and prekallikrein with cholesterol sulfate. *Thromb. Res.* **38**: 21–31.
100. Kuroki, T., T. Ikuta, M. Kashiwagi, S. Kawabe, M. Ohba, N. Huh, K. Mizuno, S. Ohno, E. Yamada, and K. Chida. 2000. Cholesterol sulfate, an activator of protein kinase C mediating squamous cell differentiation: a review. *Mutat. Res.* **462**: 189–195.
101. Okano, M., S. Kano, H. Munakata, and K. Ohtsuki. 2001. Biochemical characterization of cholesterol-3-sulfate as the sole effector for the phosphorylation of HMG1 by casein kinase 1 *in vitro*. *Biochem. Biophys. Res. Commun.* **281**: 1325–1330.
102. Woscholski, R., T. Kodaki, R. H. Palmer, M. D. Waterfield, and P. J. Parker. 1995. Modulation of the substrate specificity of the mammalian phosphatidylinositol 3-kinase by cholesterol sulfate and sulfatide. *Biochemistry*. **34**: 11489–11493.
103. Iwamori, M., Y. Iwamori, and N. Ito. 1997. Sulfated lipids as inhibitors of pancreatic trypsin and chymotrypsin in epithelium of the mammalian digestive tract. *Biochem. Biophys. Res. Commun.* **237**: 262–265.
104. Sato, J., M. Denda, J. Nakanishi, J. Nomura, and J. Koyama. 1998. Cholesterol sulfate inhibits proteases that are involved in desquamation of stratum corneum. *J. Invest. Dermatol.* **111**: 189–193.
105. Ito, M., Y. Iwamori, K. Hanaoka, and M. Iwamori. 1998. Inhibition of pancreatic elastase by sulfated lipids in the intestinal mucosa. *J. Biochem.* **123**: 107–114.
106. Iwamori, M., H. Suzuki, T. Kimura, and Y. Iwamori. 2000. Shedding of sulfated lipids into gastric fluid and inhibition of pancreatic DNase I by cholesterol sulfate in concert with bile acids. *Biochim. Biophys. Acta*. **1487**: 268–274.
107. Suzuki, Y., J. Nomura, J. Hori, J. Koyama, M. Takahashi, and I. Horii. 1993. Detection and characterisation of endogenous protease associated with desquamation of stratum corneum. *Arch. Dermatol. Res.* **285**: 372–377.
108. Suzuki, Y., J. Nomura, J. Koyama, and I. Horii. 1994. The role of proteases in stratum corneum: involvement in stratum corneum desquamation. *Arch. Dermatol. Res.* **286**: 249–253.
109. Shapiro, L. J., R. Weiss, D. Webster, and J. T. France. 1978. X-Linked ichthyosis due to steroid-sulphatase deficiency. *Lancet*. **1**: 70–72.
110. Fuchs, E. 1990. Epidermal differentiation: the bare essentials. *J. Cell Biol.* **111**: 2807–2814.
111. Rearick, J. I., and A. M. Jetten. 1986. Accumulation of cholesterol 3-sulfate during *in vitro* squamous differentiation of rabbit tracheal epithelial cells and its regulation by retinoids. *J. Biol. Chem.* **261**: 13898–13904.
112. Kiguchi, K., M. Kagehara, R. Higo, M. Iwamori, and J. DiGiovanni. 1998. Alterations in cholesterol sulfate and its biosynthetic enzyme during multistage carcinogenesis in mouse skin. *J. Invest. Dermatol.* **111**: 973–981.
113. Bouwstra, J. A., G. S. Gooris, F. E. R. Dubbelaar, and M. Ponc. 1999. Cholesterol sulfate and calcium affect stratum corneum lipid organization over a wide temperature range. *J. Lipid Res.* **40**: 2303–2312.
114. Long, S. A., P. W. Wertz, J. S. Strauss, and D. T. Downing. 1985. Human stratum corneum polar lipids and desquamation. *Arch. Dermatol. Res.* **277**: 284–287.
115. Ranasinghe, A. W., P. W. Wertz, D. T. Downing, and I. C. Mackenzie. 1986. Lipid composition of cohesive and desquamated corneocytes from mouse ear skin. *J. Invest. Dermatol.* **86**: 187–190.
116. Lin, Y. N., and M. I. Horowitz. 1980. Enzymatic sulfation of cholesterol by rat gastric mucosa. *Steroids*. **36**: 697–708.
117. Lampe, M. A., A. L. Burlingame, J. A. Whitney, M. L. Williams, B. E. Brown, E. Roitman, and P. M. Elias. 1983. Human stratum corneum lipids: characterization and regional variations. *J. Lipid Res.* **24**: 120–130.
118. Shimizu, C., H. Fuda, H. Yanai, and C. A. Strott. 2003. Conservation of the hydroxysteroid sulfotransferase SULT2B1 gene structure in the mouse: pre- and postnatal expression, kinetic analysis of isoforms, and comparison with prototypical SULT2A1. *Endocrinology*. **144**: 1186–1193.
119. Yuspa, S. H., T. Ben, and H. Hennings. 1983. The induction of epidermal transglutaminase and terminal differentiation by tumor promoters in cultured epidermal cells. *Carcinogenesis*. **4**: 1413–1418.
120. Yuspa, S. H., A. E. Kilkenny, P. M. Steinert, and D. R. Roop. 1989. Expression of murine epidermal differentiation markers is tightly regulated by restricted extracellular calcium concentrations *in vitro*. *J. Cell Biol.* **109**: 1207–1217.
121. Blumenberg, M., and M. Tomic-Canic. 1997. Human epidermal keratinocyte: keratinization processes. *EXS*. **78**: 1–29.
122. Steinert, P. M., and L. N. Marekov. 1997. Direct evidence that involucrin is a major early isopeptide cross-linked component of the keratinocyte cornified cell envelope. *J. Biol. Chem.* **272**: 2021–2030.
123. Bouwstra, J. A., F. E. R. Dubbelaar, G. S. Gooris, and M. Ponc. 2000. The lipid organisation in the skin barrier. *Acta Derm. Venerol. Suppl.* **208**: 23–30.
124. Wertz, P. W., and D. T. Downing. 1984. Cholesteryl sulfate: the major polar lipid of the horse hoof. *J. Lipid Res.* **25**: 1320–1323.
125. Hanley, K., L. Wood, D. C. Ng, S. S. He, P. Lau, A. Moser, P. M. Elias, D. D. Bikle, M. L. Williams, and K. R. Feingold. 2001. Cholesterol sulfate stimulates involucrin transcription in keratinocytes by increasing Fra-1, Fra-2, and Jun D. *J. Lipid Res.* **42**: 390–398.
126. Ransone, L. J., and I. M. Verma. 1990. Nuclear proto-oncogenes *Fos* and *Jun*. *Annu. Rev. Cell Biol.* **6**: 539–557.
127. Hanley, K., Y. Jiang, C. Katagiri, K. R. Feingold, and M. L. Williams. 1997. Epidermal steroid sulfatase and cholesterol sulfotransferase are regulated during late gestation in the fetal rat. *J. Invest. Dermatol.* **108**: 871–875.
128. Lee, J.-H., S.-I. Jang, J.-M. Yang, N. G. Markova, and P. M. Steinert. 1996. The proximal promoter of the human transglutaminase 3 gene. *J. Biol. Chem.* **271**: 4561–4568.
129. Yoshinari, K., E. V. Petrotchenko, L. C. Pedersen, and M. Negishi. 2001. Crystal structure-based studies of cytosolic sulfotransferase. *J. Biochem. Mol. Toxicol.* **15**: 67–75.
130. Negishi, M., L. G. Pedersen, E. Petrotchenko, S. Shevtsov, A. Gorokhov, Y. Kakuta, and L. C. Pedersen. 2001. Structure and function of sulfotransferases. *Arch. Biochem. Biophys.* **390**: 149–157.
131. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680.